

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



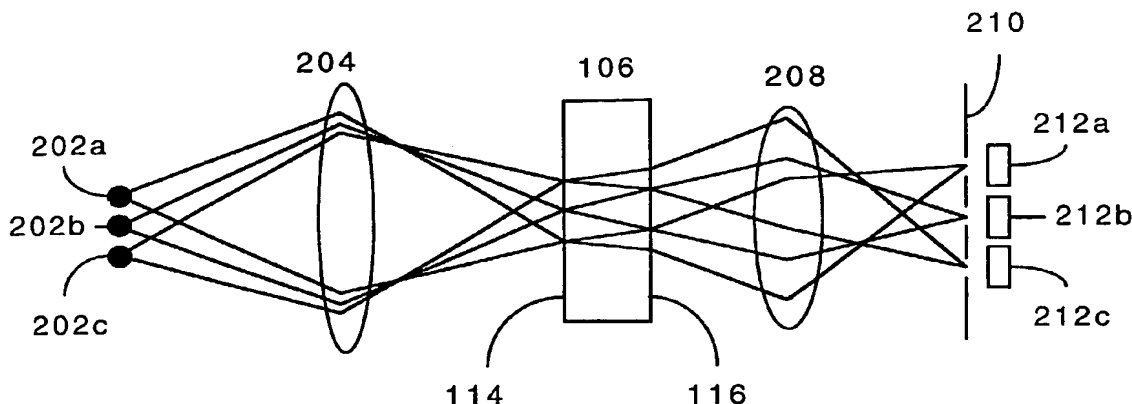
(43) International Publication Date
3 October 2002 (03.10.2002)

PCT

(10) International Publication Number
WO 02/077695 A1

- (51) International Patent Classification⁷: **G02B 21/00**
- (21) International Application Number: PCT/US02/08461
- (22) International Filing Date: 20 March 2002 (20.03.2002)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
09/814,197 21 March 2001 (21.03.2001) US
- (71) Applicant: **CDM OPTICS, INCORPORATED**
[US/US]; Suite 2110, 4001 Discovery Drive, Boulder, CO 80303-7816 (US).
- (72) Inventor: **CATHEY, Wade, Thomas, Jr.**; 228 Alpine Way, Boulder, CO 80304 (US).
- (74) Agents: **BALES, Jennifer, L.** et al.; Macheledt Bales LLP, Mountain View Plaza, 1520 Euclid Circle, Lafayette, CO 80026-1250 (US).
- (81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TT, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

(54) Title: HIGH-SPEED CONFOCAL MICROSCOPE



(57) Abstract: An improved confocal microscope utilizes an array of light sources imaged onto an object, and an array of small detectors to detect the light from each source. Cross talk between the beams of light is prevented by temporally modulating the sources at different frequencies. Light from one source is temporally modulated at a first frequency, for example in the megahertz region. A reference signal at the same frequency plus an offset frequency is also sent to the detector assigned to that source. The detected signal and the reference signal are then beat together, and heterodyne detection is used to detect only the light from the assigned source, which will beat with the reference signal and produce a signal at the offset frequency. Light from other sources beat with the reference signal to produce different frequency beat signals, which are filtered out using bandpass filters.

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HIGH SPEED CONFOCAL MICROSCOPEBACKGROUND OF THE INVENTIONFIELD OF THE INVENTION:

The present invention relates to confocal microscopes.

5

DESCRIPTION OF THE PRIOR ART:

Conventional confocal microscopes use a point source that is imaged onto the object that is to be imaged. Then, another imaging system images that part of the object through a pinhole onto a detector. By doing this, the resolution of the final image is increased because the impulse responses (or point spread functions) of each part of the system (the first imaging subsystem that images the source onto the object and the second imaging subsystem that images the object region onto the detector) are multiplied. This gives a narrower overall point spread response, and hence, better resolution.

15

The primary use of a confocal microscope, however, is to provide an image of a plane of the object without interference from other planes. That is, the confocal microscope provides an image of a slice of the object. This is accomplished because when the image of the point source is re-imaged by the second imaging subsystem onto the pinhole in front of the detector, only the light that comes from the plane being examined is focussed through the pinhole.

20

Any light from other planes spreads out rapidly and a negligible portion gets through the pinhole.

25

In order to image the whole plane being examined, both the point source and the pinhole in front of the detector must be scanned over a two dimensional array of positions corresponding to the slice. Alternatively, the object may be moved in two dimensions transverse to the optical axis.

Then, to examine another slice of the object, the point source is focussed onto

the new slice, and the scanning process is repeated. Repeating this process gives images of several slices in a volume. Alternately, the object can be moved along the optical axis.

The process of examining even a single slice of the object is very time consuming (much less many slices). Of course it works poorly with live, moving objects, because they move during the scanning time and distort the image.

Other confocal microscopes have speeded up the scanning process to some extent by using an array of micro mirrors to illuminate isolated points or by using spinning wheels with several holes in them, so that several point images can be detected at once. These point images must be far apart so that light directed at one detector pinhole does not go through a different detector pinhole when light from other planes is considered. This causes cross talk. A similar approach uses lenslet arrays to provide multiple point images. Again, the point images must be far apart. Thus, the scanning process is still very slow.

A third approach is to use structured illumination with fine detail that blurs quickly with misfocus. This approach requires multiple exposures to remove the structured illumination from the images.

A need remains in the art for faster confocal microscopes.

SUMMARY:

An object of the present invention is to provide an improved, faster, confocal microscope. The present invention accomplishes this goal by utilizing an array of light sources imaged onto the object, and an array of small detectors to detect the light from each source. The light could be visible, infrared, or ultraviolet. Cross talk between the beams of light is prevented by temporally modulating the sources at different frequencies.

Light from one source is thus temporally modulated at a first frequency, for example in the megahertz region. A reference signal at the same frequency plus a frequency offset is also sent to the detector assigned to that source. The detected signal and the reference signal are then beat together, and
5 heterodyne detection and a band pass filter are used to detect only the light from the assigned source. The light from the source will beat with the reference signal and produce a signal at the offset frequency. This signal is passed by the bandpass filter. Light from other sources, which is modulated at still different frequencies, will beat with the reference signal to produce different
10 beat signals, which can be filtered out using the band pass filter.

The detector array must be a type that can provide heterodyning, such as photodiode arrays, photo multipliers with arrays of sensor areas, and CMOS detector arrays. The array of sources must comprise near point sources, for example an array of surface emitting lasers. As an alternative, the source
15 array may comprise liquid crystal modulators illuminated with the desired illumination wavelength. The image of the source array that appears on the object can be demagnified to provide the desired resolution.

To image a different plane of the object, either the object is moved (with respect to the source array), or the lenses refocussed, as in a conventional
20 confocal microscope.

The number of distinct temporal modulation frequencies that are required is determined by the region of the detector array over which light from one source overlaps onto detectors other than the one intended for that source. This is, in turn, determined by the thickness of the object being imaged.
25 Sources may be modulated with the same frequency, so long as they are far enough apart to not overlap on the detector array.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a and 1b are side views illustrating confocal microscope imaging. In Figure 1a, a point on the object is imaged through a pinhole onto the detector. In Figure 1b, a point at a different depth on the object is not imaged through the pinhole, because of misfocus.

5 Figures 2a and 2b are side views illustrating a confocal microscope according to the present invention.

Figure 3 is a block diagram illustrating the operation of the confocal microscope of Figures 2a and 2b.

10 Figure 4 is a side view of a fluorescing confocal microscope according to the present invention.

Figure 5 is a side view illustrating a variation on the microscope of Figures 2a and 2b.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Figures 1a and 1b are side views illustrating confocal microscope imaging. In Figure 1a, a point on the object is imaged through a pinhole onto the detector (pinholes are not required if the detectors are very small. The size of the pinhole, if used, determines the lateral resolution). In Figure 1b, a point at a different depth on the object is not imaged through the pinhole, because of misfocus. Confocal microscopes use a point source 102 that is imaged by imaging system 104 onto the object 106 that is to be imaged. Then, another imaging system 108 images the illuminated part of the object through pinhole 110 onto detector 112. In Figure 1a, surface 114 of object 106 is being imaged. Thus, light focussed onto the object point on surface 114 is imaged as a point onto pinhole 110, and most of the light impinges on detector 112.

25 In Figure 1b, surface 114 is still being imaged, so light from surface 116 does

not focus to a point at pinhole 110. Consequently, only a small amount of the light from surface 116 gets through the pinhole to detector 112.

Figures 2a and 2b are side views illustrating a confocal microscope 200 according to the present invention. An array of light sources 202 are imaged
5 via lens 204 onto surface 114 of object 106, and an array of small detectors 212 behind an array of pinholes 210 detect the light from each source.

Cross talk between light from different sources is prevented by temporally modulating the sources at different frequencies. Figure 3 illustrates how this is accomplished.

10 Figure 2b illustrates what happens to light scattered from out-of-focus surface 116. This light overlaps onto a number of pinholes 212. Thus, the temporal modulation is required to permit light from the wrong source to be ignored.

Figure 3 is a block diagram illustrating the operation of the confocal microscope of Figures 2a and 2b. For simplicity, operation with two sources is shown. In practice, many more sources would be used. Light from first source
15 202a is temporally modulated at a first frequency, for example 5 megahertz, by modulation block 302a. A reference signal at the same frequency (5 MHz) is also sent to mixer and bandpass filter 304a, where it is mixed with a different offset modulating frequency 310, in this case 1MHz. The output of
20 mixer/filter 304a (at 4MHz) is provided to detector 212a.

Similarly, light from second source 202b is temporally modulated at a second frequency, for example 10 megahertz, by modulation block 302b. A reference signal at the same frequency is also sent to mixer and band pass filter 304b, where it is mixed with a modulating signal that is offset by 1MHz (for example),
25 resulting in a reference signal of 9 MHz. The output of mixer 304b (at 9MHz) is provided to detector 212b.

Signals to be mixed in a detector can be introduced to the detector either

electrically or optically. When light that is modulated at one frequency f_1 falls on a detector that also has an electrical input at a second frequency f_2 , the output of the detector has two frequency components; f_1+f_2 and f_1-f_2 .

Figure 2b shows that some light can spread and be detected by detectors other than the ones intended. For example, because of scattering from surface 116, light from source 202b can pass through the pinhole in front of detector 212a. Even though this light is reduced in intensity because less light comes through the pinhole in front of the detector, it and light from other sources contaminates the signal at detector 212a, and reduces the quality of the image. This problem is solved by modulating the light from the sources 202a and 202b at different frequencies.

The output of mixer 304a is the sum and difference of the 5 MHz modulation frequency and the 1MHz offset modulating frequency. A filter passes only the 4MHz frequency, for example, and blocks the 6MHz frequency. The 4 MHz frequency signal is introduced to the detector, and it mixes both with the direct light modulated at 5MHz from source 202a, and the undesired light that comes from source 202b via surface 116, which is modulated at 10 MHz.

The result is an electrical output of the detector that has sum and frequency components at 1MHz, 5MHz, 6MHz, 9MHz, 14MHz, and 15MHz. The bandpass filter 306a passes to output 308a only the 1MHz component which comes from interference between the desired 5MHz signal due to the modulated light from source 202a and the reference frequency from filter/mixer 304a.

Among the frequencies that are blocked are those that arise from interference between the reference frequency and the undesired 10MHz signal from light that is from the modulated source 202b. In a similar manner, only the signal from 202b is passed to output 308b, and all of the others are removed.

Those skilled in the art will appreciate that this scheme can be extended to

more than two sources, so long as the frequencies are carefully chosen. If there is sufficient distance between two sources, they can have the same modulation frequency without reducing the quality of the image. For example, for the thickness of the object 106 as shown, the light from sources 202 a and 202c do not overlap, even for the extreme value of misfocus of light coming from surface 116. Hence sources 202a and 202b could have the same modulation frequency. Thus, the same modulation frequency might be used in a pattern, e.g. every third or fourth source.

The light from object 106 need not be the same wavelength as the illumination light. For example, in fluorescence microscopy, the illuminating light excites fluorophores in the object. These then fluoresce at a wavelength that is different from the illumination. This technique is used to image specific regions of a biological image, for example, that have the fluorophores attached to them. The light from the fluorophores is modulated at the same frequency as the modulation of the illumination light. Hence, the technique described above is valid for confocal fluorescence microscopes as well.

Figure 4 is a side view of a fluorescing confocal microscope according to the present invention. When light from source 202b (for example) hits object 106, light 404 at the fluorescing wavelength is emitted. Optics 208 focus this light through pinhole 210b onto detector 212b. Because the fluorescence is caused by a modulated excitation source, the fluorescent light is also modulated at the same frequency. Hence, by using several sources that are modulated at different frequencies, the fluorescing light from different object points also has different modulating frequencies, and can be separated as shown in Figure 3.

Detector array 212 must be the type that can provide heterodyning, such as photodiode arrays, photo multipliers with arrays of sensor areas, or CMOS detector arrays. CCD arrays, which ignore temporal modulation, cannot be used.

Source array 202 must comprise approximately point sources, for example an array of surface emitting lasers. As an alternative, the source array may comprise liquid crystal modulators illuminated with the desired illumination wavelength. The light can be infrared, visible, or ultraviolet. Ultraviolet light is often used for high resolution, and also for fluorescence microscopy.

The image of the source array that appears on object 106 can be demagnified to provide the desired resolution. To image a different plane of object 106, either object 106 is moved with respect to source array 202, or imaging system 204 is refocussed.

The spacing for the pinholes for the detector array and the spacing of the detectors need not be the same. An imaging system between the two can be used to image the light that passes through the array of pinholes that are in front of the detectors onto the array of detectors.

Figure 5 is a side view illustrating a variation on the microscope of Figures 2a and 2b. Additional optics 502 are placed between pinhole array 210 and detector array 512, in order to redirect the light from the pinholes onto the desired detectors. This is useful if the detector array must have a wider spacing than the array of sources.

What is claimed is:

CLAIMS

1. A confocal microscope comprising:

an array of light sources;

means for imaging the array of light sources onto a plane of the object;

5 an array of detectors, each detector corresponding to a source;

means for imaging light from the object onto the array of detectors; and

means for removing cross talk between light from sources including, for each source-

10 means for temporally modulating light from the source at a specified frequency,

means associated with the corresponding detector for beating the signal detected by the detector with a signal at the modulating frequency plus a different offset frequency, and

15 a low pass filter for filtering out any signal at a frequency significantly different than the offset frequency.

2. The confocal microscope of claim 1, wherein the light sources emit visible light.

3. The confocal microscope of claim 1, wherein the light sources emit infrared light.

4. The confocal microscope of claim 1, wherein the light sources emit ultraviolet light.

5. The confocal microscope of claim 1, wherein the beating means operates

on optical signals.

6. The confocal microscope of claim 1, wherein the beating means operates on electrical signals.

7. The confocal microscope of claim 1, wherein the object includes fluorophores, and wherein the fluorophores fluoresce at a fluorescing frequency when impinged upon by a light source, and
wherein the detectors detect light at the fluorescing frequency.

8. The confocal microscope of claim 1, further including an array of pinholes, corresponding to the array of detectors and located between the object and the array of detectors adjacent, for enhancing lateral resolution.

9. A confocal microscope comprising:

an array of light sources;

means for imaging the array of light sources onto a plane of the object;

an array of detectors, each detector corresponding to a

5 source;

an array of pinholes corresponding to the array of

detectors and located between the object and the array of

detectors;

means for imaging light from the object onto the array of pinholes;

10 optics between the pinhole array and the detector array for

focussing light from the pinhole array onto the detector array;

and

means for removing cross talk between light from sources

including, for each source-

15 means for temporally modulating light from the source at a

specified frequency,

means associated with the corresponding detector for beating the signal

detected by the detector with a signal at the modulating frequency plus

a different offset frequency, and

20 a low pass filter for filtering out any signal at a frequency

significantly different than the offset frequency.

10. The confocal microscope of claim 9, wherein the light sources emit visible light.

11. The confocal microscope of claim 9, wherein the light sources emit infrared light.

12. The confocal microscope of claim 9, wherein the light sources emit ultraviolet light.

13. The confocal microscope of claim 9, wherein the beating means operates on optical signals.

14. The confocal microscope of claim 9, wherein the beating means operates on electrical signals.

15. The confocal microscope of claim 9, wherein the object includes fluorophores, and wherein the fluorophores fluoresce at a fluorescing frequency when impinged upon by a light source, and wherein the detectors detect light at the fluorescing frequency.

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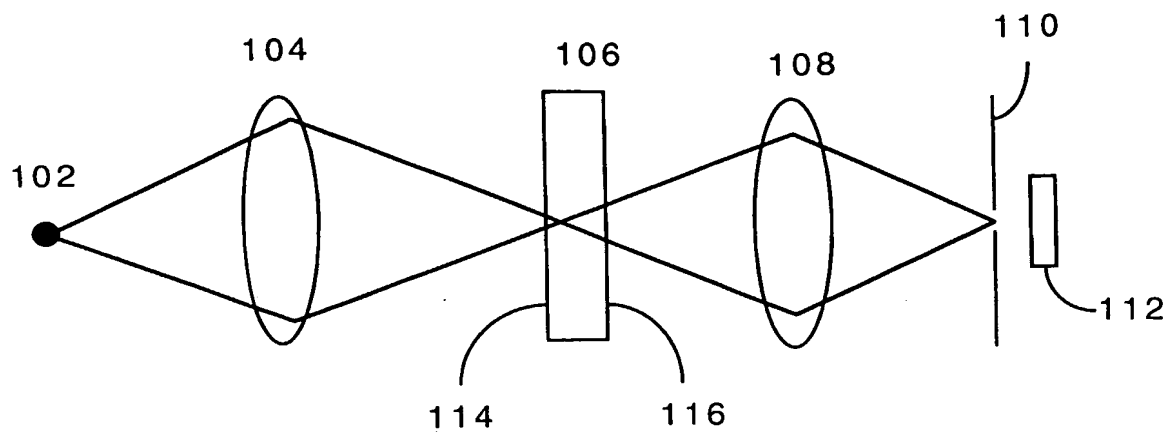


Figure 1a

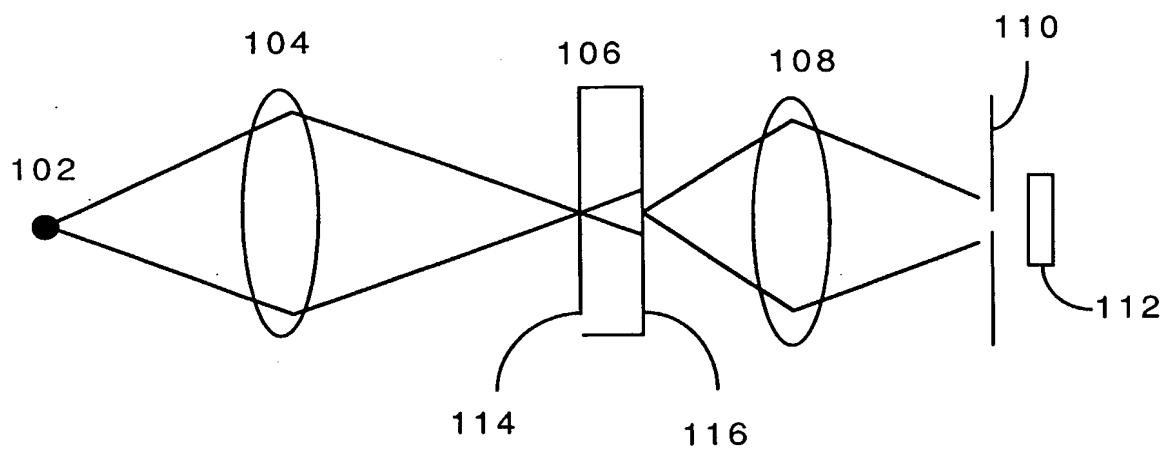


Figure 1b

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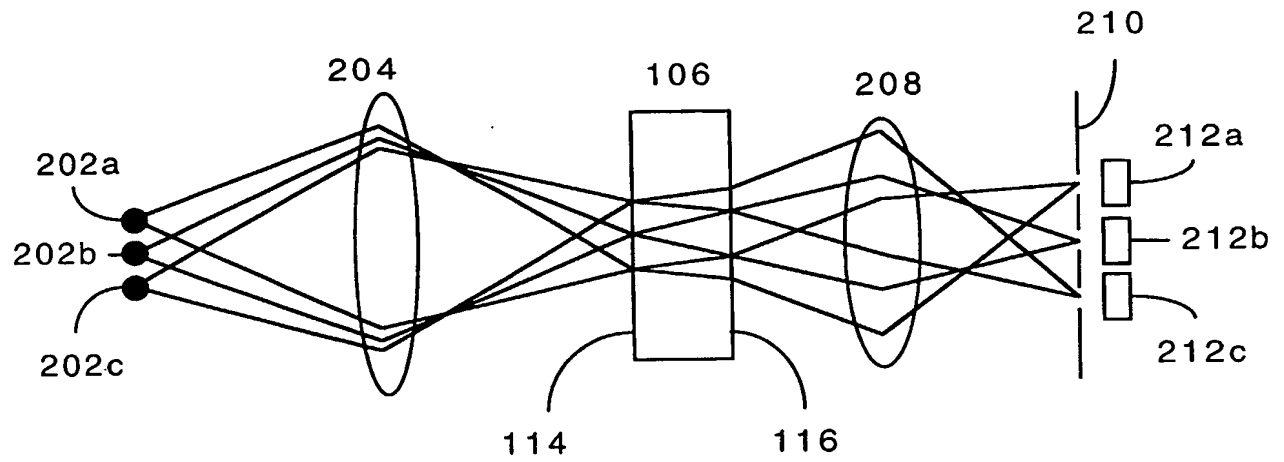


Figure 2a

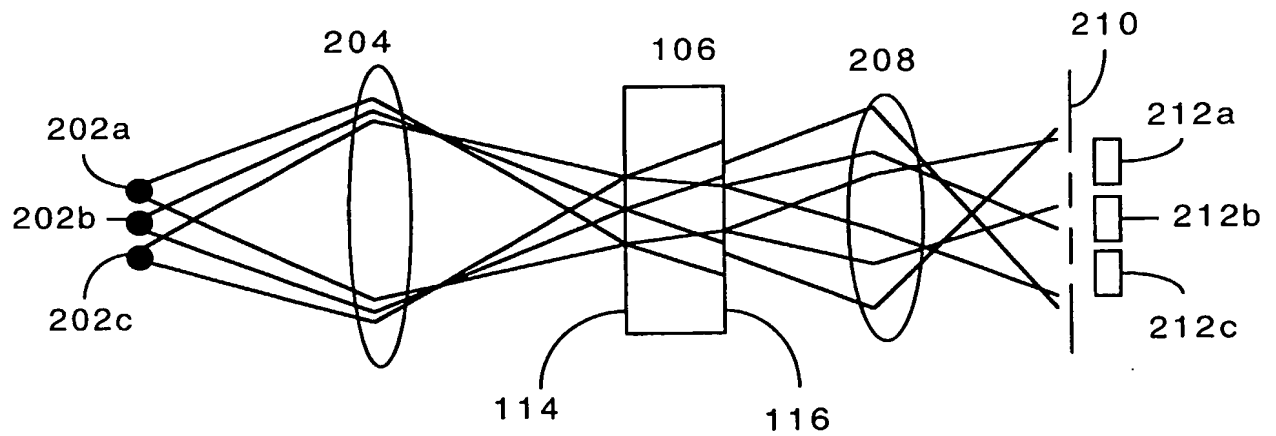


Figure 2b

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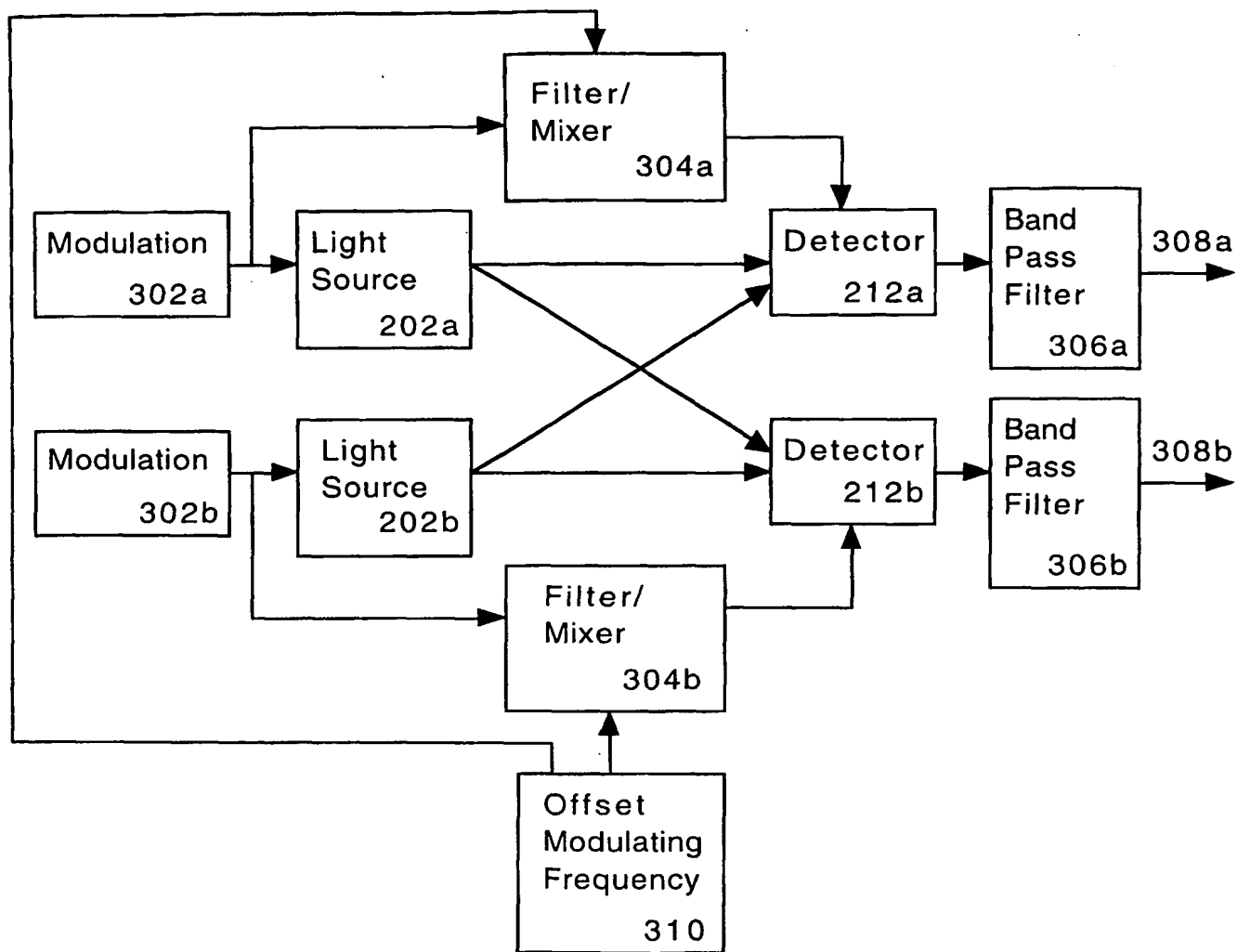


Figure 3

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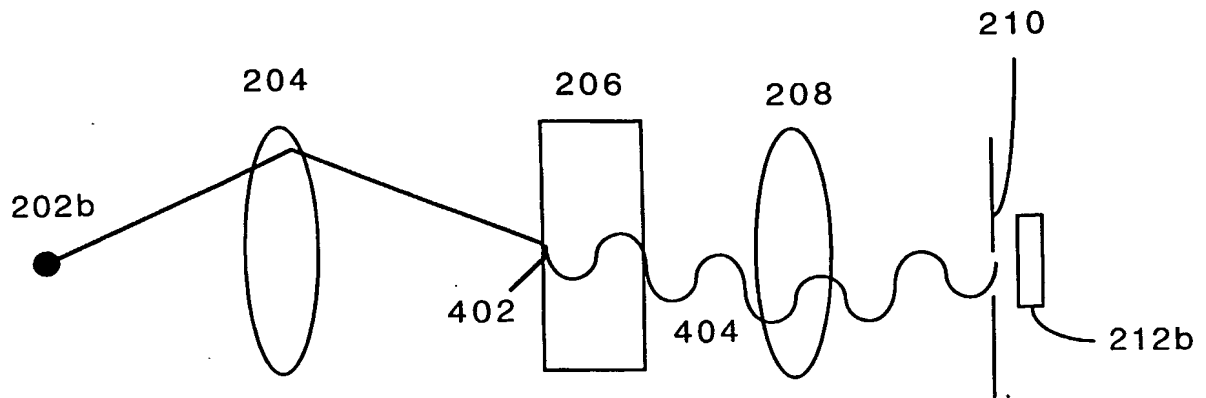


Figure 4

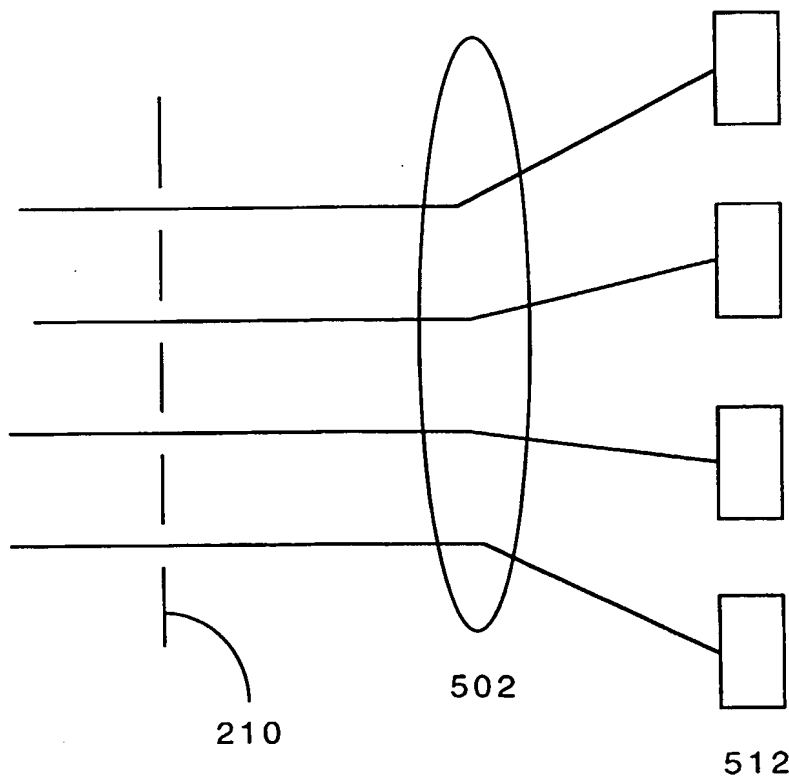


Figure 5

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/08461

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 G02B21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G02B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, INSPEC, COMPENDEX, IBM-TDB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	US 6 248 988 B1 (KRANTZ MATTHIAS C) 19 June 2001 (2001-06-19) abstract; claims 39,128,130; figures 1,2A,2B,10A,10B,18A,18B column 3, line 47 -column 4, line 28 column 9, line 4 - line 40 ---	1-15
A	FUKANO T ET AL: "GEOMETRICAL CROSS-SECTIONAL IMAGING BY A HETERODYNE WAVELENGTH-SCANNING INTERFERENCE CONFOCAL MICROSCOPE" OPTICS LETTERS, OPTICAL SOCIETY OF AMERICA, WASHINGTON, US, vol. 25, no. 8, 15 April 2000 (2000-04-15), pages 548-550, XP000951941 ISSN: 0146-9592 the whole document ---	1-15
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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Date of the actual completion of the international search

5 July 2002

Date of mailing of the international search report

16/07/2002

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Michel, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 02/08461

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SCHMITT J M ET AL: "An optical coherence microscope with enhanced resolving power in thick tissue" OPTICS COMMUNICATIONS, NORTH-HOLLAND PUBLISHING CO. AMSTERDAM, NL, vol. 142, no. 4-6, 15 October 1997 (1997-10-15), pages 203-207, XP004093980 ISSN: 0030-4018 paragraphs '02.2!', '0003! -----	1-15
A	SHATALIN S V ET AL: "Polarisation contrast imaging of thin films in scanning microscopy" OPTICS COMMUNICATIONS, NORTH-HOLLAND PUBLISHING CO. AMSTERDAM, NL, vol. 116, no. 4, 1 May 1995 (1995-05-01), pages 291-299, XP004011523 ISSN: 0030-4018 page 296, line 4 -page 297, line 25 -----	1-15
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A	US 5 465 147 A (SWANSON ERIC A) 7 November 1995 (1995-11-07) the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 02/08461

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